



# CHARACTERIZATION OF CHOLINESTERASE ACTIVITY IN TISSUE OF ADULT AND LARVAL GRASS SHRIMP (*PALAEMONETES PUGIO*)

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## ABSTRACT

The grass shrimp (*Palaemonetes pugio*) is a common inhabitant of salt marshes along the Atlantic coast of the US. Along the South Carolina coast, agricultural fields are often located adjacent to grass shrimp habitats, so all life history stages of these organisms face a significant risk of exposure to various pesticides during the growing season. Organophosphorus insecticides (OPs) are of particular concern in terms of the nontarget exposure of grass shrimp. These compounds produce toxicity by inhibiting cholinesterase (ChE) enzymes in neural tissues. While earlier studies have shown that cholinesterases in grass shrimp are inhibited by OPs, the substrate specificity of these enzymes has not been well established. In this study, ChE activity was measured in the tissue of adult and larval grass shrimp. Multiple substrates, acetylthiocholine (ACTH), propionylthiocholine (PRTH) and butyrylthiocholine (BUTH), and two selective inhibitors, ISO-OMPA and eserine, were utilized to characterize ChE activity. Adult grass shrimp were found to have an average ChE level with the ACTH substrate of 49.12 nmol/mgP/min while ChE levels with the BUTH substrate averaged significantly lower at 1.42 nmol/mgP/min. Newly hatched larvae ChE levels ranged from 210.42 nmol/mgP/min with the ACTH substrate to 8.52 nmol/mgP/min with the BUTH substrate. In adult and larval tissue, the inhibitor ISO-OMPA was not found to have a significant reduction on ChE activity while eserine inhibited all activity.



## INTRODUCTION

- Grass shrimp** (*Palaemonetes pugio*) are common inhabitants of salt marshes along the Atlantic Coast of the US.
  - Pesticides are a crucial concern in coastal areas where agriculture and increasing resort development (urbanization, golf courses) impact the surrounding estuaries and marshes.
  - Grass shrimp inhabiting these surrounding areas thus risk exposure to pesticides and are affected by pesticide runoff (Key et al., 1998).
- Organophosphorus insecticides** (OPs) are the pesticide of choice in many agricultural and resort development situations.
  - OPs have been implicated in fish kills and are commonly detected in aquatic biota (Pait et al., 1992).
  - OPs produce toxicity by inhibiting the cholinesterase enzyme (ChE) in the nervous system.
- ChEs** in grass shrimp adults and larvae have been shown to be inhibited by OPs (Key, 1995).
  - Research with fish has found specific ChEs: acetylcholinesterase in brain and acetylcholinesterase and butyrylcholinesterase in muscle (Fulton et al., 1998).
- Objectives** of this research were the following:
  - Determine baseline ChE activity for adult and newly hatched larval grass shrimp utilizing three different substrates.
  - Characterize the ChE activity in adult and larval grass shrimp using the three substrates and two ChE inhibitors.

## MATERIALS and METHODS

### 1. Test Animal

- Adult *P. pugio* were collected from Leadenwah Creek (N 32°36'12" ; W 80°07'00"), a tidal tributary of the North Edisto River estuary in South Carolina.
- Adult shrimp were acclimated in 76-L tanks at 25°C, 20‰ salinity and 12-h light:12-h dark cycle. Shrimp were fed TetraMin® Fish Flakes and Artemia.
- To collect larvae (zoea), gravid females were placed in brooding traps to allow larvae to hatch and escape without interference. Larvae from at least 10 females were pooled for all tests.

### 2. Substrates

- Acetylthiocholine (ACTH) - In vertebrates the hydrolysis of this substrate is catalyzed by both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).
- Propionylcholinesterase (PRTH) - Hydrolysis of this substrate is catalyzed by ChEs in both vertebrates and invertebrates.
- Butyrylcholinesterase (BUTH) - In vertebrates the hydrolysis of this substrate is preferentially catalyzed by BChE.

### 3. Inhibitors

- Eserine (ESE) - Inhibits ChEs.
- ISO-OMPA - Inhibits BChE.

### 4. ChE Assay

- One whole adult grass shrimp was used for each adult sample. Approximately 30 whole newly hatched larvae were used for each larval sample.
- For each sample 75 µl of sample homogenate were added to 23 test tubes and used as follows:
  - 7 tubes with 10 µl of 75 mM ACTH substrate
    - 1 tube received 15 µl of 10<sup>-3</sup>M ESE
    - 2 tubes received 15 µl of 10<sup>-3</sup>M ISO-OMPA
    - 2 tubes received 15 µl of 10<sup>-3</sup>M ISO-OMPA
    - 2 tubes received 15 µl of 100% ethanol (control)
  - 7 tubes with 10 µl of 150 mM PRTH substrate (each treated as above)
  - 7 tubes with 10 µl of 150 mM BUTH substrate (each treated as above)
  - 2 tubes for protein analysis
- ChE activity was measured using a modification of the method described by Key et al. (1998). ChE activity was calculated as nmol product formed/mg Protein/min.

### 5. Data Analysis

- ANOVA was used to determine if significant group differences ( $p \leq 0.05$ ) existed. Scheffe's multiple comparison test was used to determine significant differences among the treatments.

FIGURE 1. ChE activity vs. three substrates in adult grass shrimp.

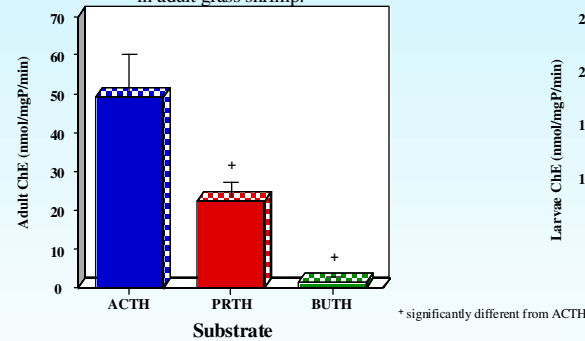


FIGURE 2. ChE activity vs. three substrates in larval grass shrimp.

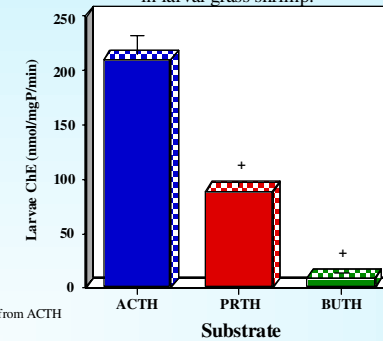


FIGURE 3. ChE activity with ACTH substrate vs. ISO-OMPA and ESE in adult grass shrimp.

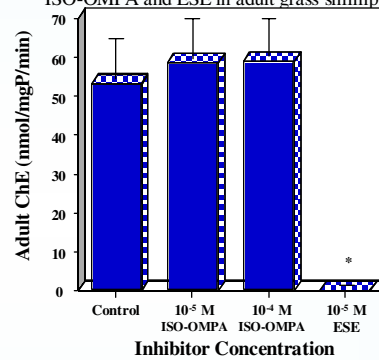


FIGURE 4. ChE activity with ACTH substrate vs. ISO-OMPA and ESE in larval grass shrimp.

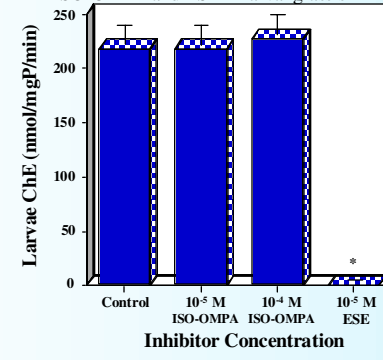


FIGURE 5. ChE activity with PRTH substrate vs. ISO-OMPA and ESE in adult grass shrimp.

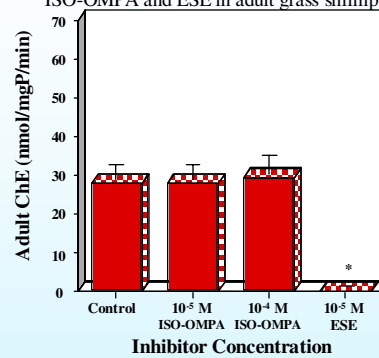


FIGURE 6. ChE activity with PRTH substrate vs. ISO-OMPA and ESE in larval grass shrimp.

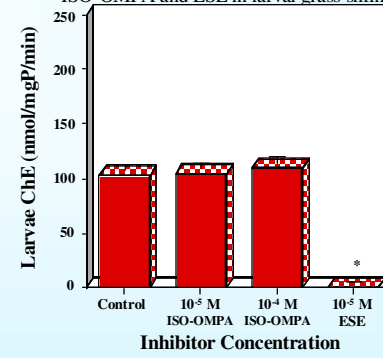


FIGURE 7. ChE activity with BUTH substrate vs. ISO-OMPA and ESE in adult grass shrimp.

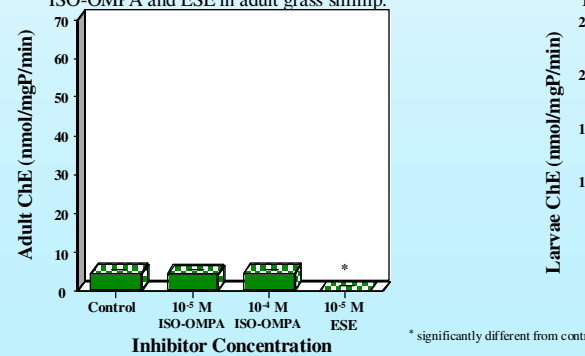
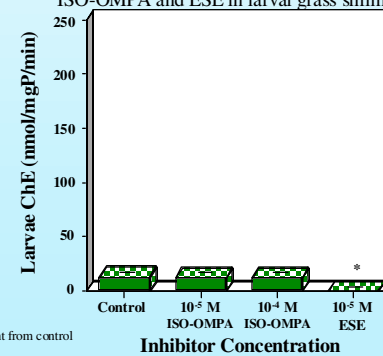


FIGURE 8. ChE activity with BUTH substrate vs. ISO-OMPA and ESE in larval grass shrimp.



## RESULTS

### 1. Substrates

- For both adult and larvae, whole body assays showed similar results with the three substrates (FIGURES 1 and 2).
  - Highest ChE activity was obtained with ACTH: 49.12 nmol/mgP/min in adults 210.42 nmol/mgP/min in larvae
  - Lowest ChE activity occurred with BUTH: 1.42 nmol/mgP/min in adults 8.52 nmol/mgP/min in larvae
  - ChE activity obtained with PRTH was >45% of adult ACTH value and >42% of larvae ACTH value.
- Overall larval activity was consistently higher than adult activity.

### 2. Inhibitors

- Hydrolysis of ACTH was not inhibited in either adult or larval tissues by ISO-OMPA at 10<sup>-5</sup> or 10<sup>-4</sup> M (FIGURES 3 and 4).
- Hydrolysis of ACTH was completely inhibited by ESE (10<sup>-5</sup> M) in both adult and larval tissues (FIGURES 3 and 4).
- Hydrolysis of PRTH was not inhibited in either adult or larval tissues by ISO-OMPA at 10<sup>-5</sup> or 10<sup>-4</sup> M (FIGURES 5 and 6).
- Hydrolysis of PRTH was completely inhibited by ESE (10<sup>-5</sup> M) in both adult and larval tissues (FIGURES 5 and 6).
- Hydrolysis of BUTH was not inhibited in either adult or larval tissues by ISO-OMPA at 10<sup>-5</sup> or 10<sup>-4</sup> M (FIGURES 7 and 8).
- Hydrolysis of BUTH was completely inhibited by ESE (10<sup>-5</sup> M) in both adult and larval tissues (FIGURES 7 and 8).

## CONCLUSIONS

- ChE activity in both adult and larval grass shrimp tissues displayed a distinct preference for ACTH over either PRTH or BUTH.
- ChE activity in tissues from both adults and larvae was lowest for BUTH.
- ChE activity versus PRTH was similar to that reported for crab ganglion and catfish brain tissue (Habig et al., 1988 and Anderson et al., 1978).
- ChE activity in each of the tissues was sensitive to ESE but insensitive to ISO-OMPA.
- These findings suggest the absence of BChE-like activity in these crustacean tissues.
- The response of ChE activity in grass shrimp appears to be similar to AChE in vertebrates.

## REFERENCES

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